

WHAT IS CLAIMED IS:

1. A recombinant α -L-iduronidase enzyme or biologically active fragments or mutein thereof with a purity of greater than 99%.

2. The recombinant α -L-iduronidase enzyme or biologically active fragments or mutein thereof of Claim 1 with a specific activity greater than about 240,000 units per milligram protein.

3. A method of producing human α -L-iduronidase of Claim 1, comprising the steps of:

(a) preparing a seed train of cells transformed with nucleic acids encoding for inoculation into a bioreactor;

(b) preparing a mixture containing macroporous microcarriers by washing and autoclaving said microcarriers in phosphate buffered saline, combining said microcarriers with growth medium and fetal bovine serum, and pumping said microcarrier mixture into said bioreactor;

(c) inoculating and incubating said cells in said bioreactor under control of pH, dissolved oxygen and perfusion; and

(d) harvesting cells when cell density reaches about 10^6 .

4. A method of preparing seed train of said cells of Claim 3 for mass production, comprising:

(a) washing and resuspending an aliquot of working cell bank CHO cells 2.131 in culture medium containing protein-free medium with supplementation of 7.6 mg/L thymidine, 13.6 mg/L hypoxanthine, 375 μ g/mL G418 and 5% fetal bovine serum;

(b) incubating said cell suspension for two to three days at 37°C and 5% carbon dioxide in three 225 cm²-flasks;

(c) splitting said cell suspension by adding the cells sequentially to one 1-liter spinner flask, two 3-liter flasks, and four 8-liter flasks;

(d) rotating said cell suspension at 50 revolutions per minute, followed by increasing inoculum volume by incubating and subculturing cells to a
5 final cell density of about 2.0×10^5 to 2.5×10^5 .

5. A method of purifying of α -L-iduronidase of Claim 1 to greater than about 99% purity, comprising the steps of:

(a) harvesting and filtering fluid obtained from a culture of cells transformed with nucleic acids encoding said human α -L-iduronidase;

10 (b) adjusting the pH of the fluid to an acidic pH, followed by filtration through a 0.2 micron to 0.54 micron filter;

(c) passing the fluid through a blue sepharose FF column to capture said human recombinant α -L-iduronidase;

15 (d) passing the fluid through a copper chelating sepharose column to remove contaminating CHO proteins;

(e) passing the fluid through a phenyl sepharose column to reduce residual leached Cibacron blue dye and copper ions carried over from previous columns; and

(f) concentrating and diafiltering the purified α -L-iduronidase.

20 6. The method of Claim 5, wherein said blue sepharose FF column is used to purify said human α -L-iduronidase seven to ten fold.

7. The method of Claim 5, wherein said method comprises using 10% glycerol in all buffers to increase the quantitative recovery of said human α -L-iduronidase.